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XcelGen | CP Plant gDNA Mini Kit

User Guide

Cat No: XG2621-01
XcelGen
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Revised Protocol

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Introduction

The CP Plant DNA mini Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from fresh and dried plant tissue samples rich in polysaccharides or lower DNA contents. Up to 100 mg of wet tissue (or 30 mg dry tissue) can be processed within short time. The system combines the reversible nucleic acid-binding properties of the matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. Purified DNA is suitable for PCR, restriction digestion and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the CP Plant DNA Kit for the first time, please read this booklet to become familiar with the procedures. This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of XcelGen matrix. Samples are homogenized and lysed in a high salt Buffer containing CTAB and extracted with chloroform to remove polysaccharides and other components that interfere with many DNA isolations and downstream applications. Binding conditions are then adjusted and DNA is further purified using DNA spin columns. In this way salts, proteins and other contaminants are removed to yield high quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification and hybridization techniques.

Storage and Stability

All components of the CP plant DNA kit are stable for at least 12 months when stored at 22°C-25°C. RNase A should be stored at 4°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer CP1 and Buffer CP2 and Buffer BL. It is possible to dissolve such deposits by warming the solution at 50°C, though we have found that they do not interfere with overall performance.

Kit Contents

Product	XG2621-00	XG2621-01
DNA Columns	4	50
2 ml collection tubes	8	100
Buffer BL	1.8 ml	20 ml
Buffer CP1	3.5 ml	40 ml
Buffer CP2	1 ml	10 ml
DNA Wash Buffer	2 ml	15 ml
RNAse A 20mg/ml	30 µl	270 µl
Elution Buffer	1.5 ml	15 ml
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Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important:

Dilute Wash Buffer Concentrate with ethanol as follows and store at room temperature. Add 8ml (XG22611-00) or 60ml (XG2611-01) absolute (96%-100%) ethanol to each bottle.

Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens.

A. Dry Specimens (Page 4)	For processing ⩽ 50 mg powdered tissue. DNA yields range from 10 µg to 50 µg per 100 mg dry tissue.
B. Fresh/Frozen Specimens (Page 7)	For processing ⩽ 100 mg fresh (or frozen) tissue. DNA yield is similar to A.

CP Plant gDNA Mini Kit Protocol

A. Dry Specimens

Materials supplied by user:

- Microcentrifuge capable of at least 14,000g.
- Nuclease-free 1.5 ml and 2.0 ml centrifuge tubes.
- Chloroform:isoamyl alcohol (24:1).
- Waterbath equilibrated to 65°C.
- Equilibrate sterile ddH_2O or Elution Buffer (pH 8.5) at 65°C.
- Absolute (96%-100%) ethanol.
- Optional: β-mercaptoethanol.

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA. Yields are usually sufficient for several tracks on a Southern blot for RFLP mapping. Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45°C oven, powdered, and stored dry at room temperature. To prepare dried samples, place ~50 mg of dried tissue into a 2.0 ml microfuge tube and grind using a pellet pestle. Disposable Knots pestles work well and are available from XcelGen. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield. Process in sets of four to six tubes until Step 2 before starting another set.

1. Add **400µl Buffer BL** into the spin column, incubate at room temperature for 2 minutes, centrifuge at 12,000 rpm for 2 minutes and discard the flow through. The column is ready and will work well for binding DNA.
2. To 10-50 mg powdered dry tissue, add **700 µl Buffer CP1** in a 2.0 ml microfuge tube.

   Optional: *Add 6µl β-mercaptoethanol and vortex vigorously to mix. Make sure to disperse all clumps.*
3. Incubate at 65°C for 15 min. Mix sample twice during incubation by inverting tube.

   Optional: *If necessary, add* ***5µl of RNase A*** *into the lysate before incubation to remove the RNA.*

4. Add **600µl chloroform/Isoamyl alcohol (24:1)** and vortex to mix. Centrifuge at 10,000 g for 10 min.
5. Carefully aspirate 300µl supernatant to a new 1.5ml microfuge tube making sure not to disturb the pellet or transfer any debris.
6. Adjust binding conditions of the sample by adding **150µl Buffer CP2** followed by **300µl absolute ethanol** and vortex to obtain a homogeneous mixture. A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.

   Note: *This point to start the optional vacuum/spin protocol.* *(See Page 10 for details.)*
7. Apply the entire sample (including any precipitate that may have formed) to a DNA column placed in a 2.0 ml collection tube (supplied). Centrifuge the column at 10,000g for 1 min to bind DNA. Discard both the 2.0 ml collection tube and the flow-through liquid.
8. Transfer column to a second collection tube and wash by adding **650µl DNA Wash Buffer** diluted with absolute (96%-100%) ethanol. Centrifuge at 10,000g for 1 min and discard the flow-through liquid. Reuse the collection tube in next step below.

   Note: *DNA Wash Buffer must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.*
9. Repeat wash step with an additional **650µl DNA Wash Buffer**. Centrifuge at 10,000g for 1 min. Discard flow-through and collection tube.
10. Place the column into a new collection tube and centrifuge empty column, with the lid open, for 2 min at maximum speed to dry. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
11. Transfer column to a clean 1.5ml tube. Apply **100µl Elution Buffer** pre-warmed to 65°C and immediately Centrifuge at maximum speed for 1 min to elute DNA. Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 200µl of Buffer for elution is not recommended.
12. Repeat Step 11 with an additional **100µl of Elution Buffer**. This may be performed using another 1.5 ml tube to maintain a higher DNA concentration in the first elute.

    Tip: *To increase DNA concentration add Buffer and incubate the column at 60°C-70°C for 5 min before elution.*

    Total DNA yields vary depending on type and quantity of sample. Typically, 10-50 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 50 mg dried tissue.

B. Fresh/Frozen Specimens

Materials supplied by user:

- Microcentrifuge capable of 14,000g
- Nuclease-free 1.5 ml and 2.0 ml microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile ddH_2O water or Elution Buffer (pH 8.5) at 65°C.
- Absolute (96%-100%) ethanol
- Chloroform:isoamyl alcohol (24:1)
- Liquid nitrogen for freezing/disrupting samples
- Optional: β-mercaptoethanol

Note: *Use extreme caution when handling liquid nitrogen.*

This protocol is suitable for most fresh or frozen tissue samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to 200 mg. Best results are obtained with young leaves or needles. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples collect tissue in a 1.5ml or 2ml microfuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pellet pestles. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until cleaning. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.

1. Add **400µl Buffer BL** into the spin column, incubate at room temperature for 2 minutes, centrifuge at 12,000 rpm for 2 minutes and discard the flow through. The column is ready and will work well for binding DNA.
2. Collect ground plant tissue (start with 100mg) in a 2.0 ml microfuge tube and immediately add **500µl Buffer CP1**.

   Optional: *Add 6µl β-mercaptoethanol and vortex vigorously. Make sure to disperse all clumps.*

3. Incubate at 65°C for 15 min. Mix sample twice during incubation by inverting tube. ***Optional:*** *If necessary, add* ***5µl of RNase A*** *into the lysate before incubation to remove the RNA.*
4. Add **800µl chloroform/Isoamyl alcohol (24:1)** and vortex to mix. Centrifuge at 10,000g for 5 min. Carefully aspirate 300µl supernatant to a new 1.5ml microfuge tube making sure not to disturb the pellet or transfer any debris.
5. Adjust binding conditions of the sample by adding **150µl Buffer CP2** followed by 300µl absolute ethanol and vortex to obtain a homogeneous mixture. A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.
6. Apply the entire sample (including any precipitate that may have formed) to a DNA column placed in a 2.0ml collection tube (supplied). Centrifuge the column at 10,000g for 1 min to bind DNA. Discard both the 2.0 ml collection tube and the flow-through liquid.

   Note: *This point to start the optional vacuum/spin protocol.* *(See Page 10 for details.)*
7. Transfer column to a second collection tube and wash by adding **650µl DNA Wash Buffer** diluted with absolute (96%-100%) ethanol. Centrifuge at 10,000g for 1 min and discard the flow-through liquid. Reuse the collection tube in next step below.

   Note: *DNA Wash Buffer must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.*
8. Repeat wash step with an additional **650µl DNA Wash Buffer**. Centrifuge at 10,000g for 1 min. Discard flow-through and collection tube.
9. Place the column into a new collection tube and Centrifuge empty column 2 min at maximum speed to dry. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
10. Transfer column to a clean 1.5 ml tube. Apply **100µl Elution Buffer**, pre-warmed to 65°C and incubate at room temperature for 1 min. Centrifuge at 14,000g for 1 min to elute DNA. Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 200µl of Buffer for elution is not recommended.
11. Repeat Step 11 with an additional **100µl of Elution Buffer**. This may be performed using another 1.5 ml tube to maintain a higher DNA concentration in the first elute.

    Tip: *To increase DNA concentration add Buffer and incubate the column at 60°C-70°C for 5 min before elution.*

    Total DNA yields vary depending on type and quantity of sample. Typically 10-50 µg DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 100mg of fresh / frozen sample.

Vacuum/Spin Protocol for CP Plant gDNA Mini Kit

Note: *Please read through previous section of this book before using this protocol.*

1. Prepare wet or dry samples by following the standard Protocol in previous sections until loading DNA/CP2/Ethanol mixture to DNA column.
2. Prepare the vacuum manifold according to manufacturer's instruction and connect the spin column to the manifold.
3. Load the DNA/CP2/Ethanol solution to the column.
4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
5. Wash the column by adding **650µl DNA Wash Buffer**, draw the wash Buffer through the column by turn on the vacuum source. Repeat this step with another **650µl DNA Wash Buffer**.
6. Assemble the column into a 2ml collection tube and transfer the column to a micro centrifuge. Spin 1 min to dry the column.
7. Place the column in a clean 1.5ml microcentrifuge tube and add 100µl Elution Buffer. Centrifuge at maximum speed for 1 min to elute DNA.

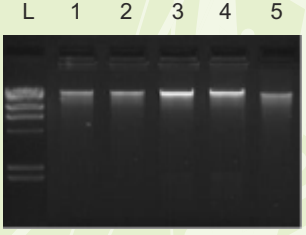

Fig: Agarose gel analysis of Plant gDNA purified with XcelGen C P Plant gDNA mini Kit.

Lane L: Hind III DNA ladder
Lane 1: gDNA Isolated from Banana
Lane 2: gDNA Isolated from Maize
Lane 3: gDNA Isolated from Mango
Lane 4: gDNA Isolated from Rubber
Lane 5: gDNA Isolated from Chikoo

Troubleshooting Guide

Problem	Possible reason	Suggested Improvements
Clogged well	Carry-over of debris.	Following precipitation with chloroform / Isoamyl alcohol, make sure no particulate material is transferred.
	DNA pellet not completely dissolved before applying sample to column.	Ensure that DNA is dissolved in water before adding Buffer CP2 and ethanol. This may need repeated incubation at 65°C and vortexing.
	Sample too viscous	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers CP1 and CP2 and use two or more columns per sample.
Low yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a new homogeneous powder adding Buffer CP1.
	Poor lysis of tissue	Decrease amount of starting material or increase amount of Buffer CP1, chloroform: isoamylalcohol, Buffer CP2.
	DNA remains bound to column.	Increase elution volume to 200 µl and incubate on column at 65°C for 5 min before centrifugation.
	DNA washed off.	Dilute Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	Salt carry-over.	DNA Wash Buffer must be at room a fine homogeneous powder adding temperature.
	Ethanol carry-over	Following the second wash spin, ensure that the column is dried by centrifuging 2 min at maximum speed.

Related Products

1. Plant gDNA mini kit (XG2611-01)
2. DNA Gel/PCR Purification Mini kit (XG3511-01/XG3514)
3. Premix Taq V2.0 (XG334A)
4. Taq DNA Polymerase (XG00007-1000/ XG00007-10000)
5. Pfu DNA Polymerase (XG00021-100/ XG00021-500)
6. dNTP Mixture 10 mM each (XG0056)
7. 100 bp DNA Ladder (XGM250)
8. 1 kb DNA Ladder (XGM1k)
9. Agarose Hipure(LE) (XGA-100)

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human. This product is warranted to perform as described in its labeling and in XcelGen's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by XcelGen. XcelGen's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of XcelGen, to replace the products, XcelGen shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

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